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Riber, Leise

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Salmonella genes involved in attachment on carcass surface and pork meat contamination

Leise Riber & Jeffrey Hoorfar
National Food Institute, DTU, Copenhagen, Denmark
E-mail: leiri@food.dtu.dk
Collaborators: KU-LIFE, DK & IFR, UK

BACKGROUND

Human infections caused by *Salmonella* from contaminated food is a common problem. Consequently, there is a need for optimizing food production environment in order to reduce any threat to human health caused by the food chain.

AIM

Establish a new approach to identify and use genes of *Salmonella* involved in the attachment on carcass surfaces and in pork chain contamination. With the design of a new meat surface model, we will analyze a number of meat and non-meat isolates for their ability to express the attachment genes. Subsequently, we will look at the

time-course of the expression profile of attachment, virulence and lag-phase genes to see if specific probes can be designed to be used for "early-warning" assays. This may give us an idea of what kind of food products and production environments that facilitate expression of lag-phase genes.

STRATEGY

- Identify genes involved in attachment to meat
- Study the influence of the "history" of *Salmonella* on attachment potential
- Measure the expression of selected genes over time for *Salmonella* on a meat surface

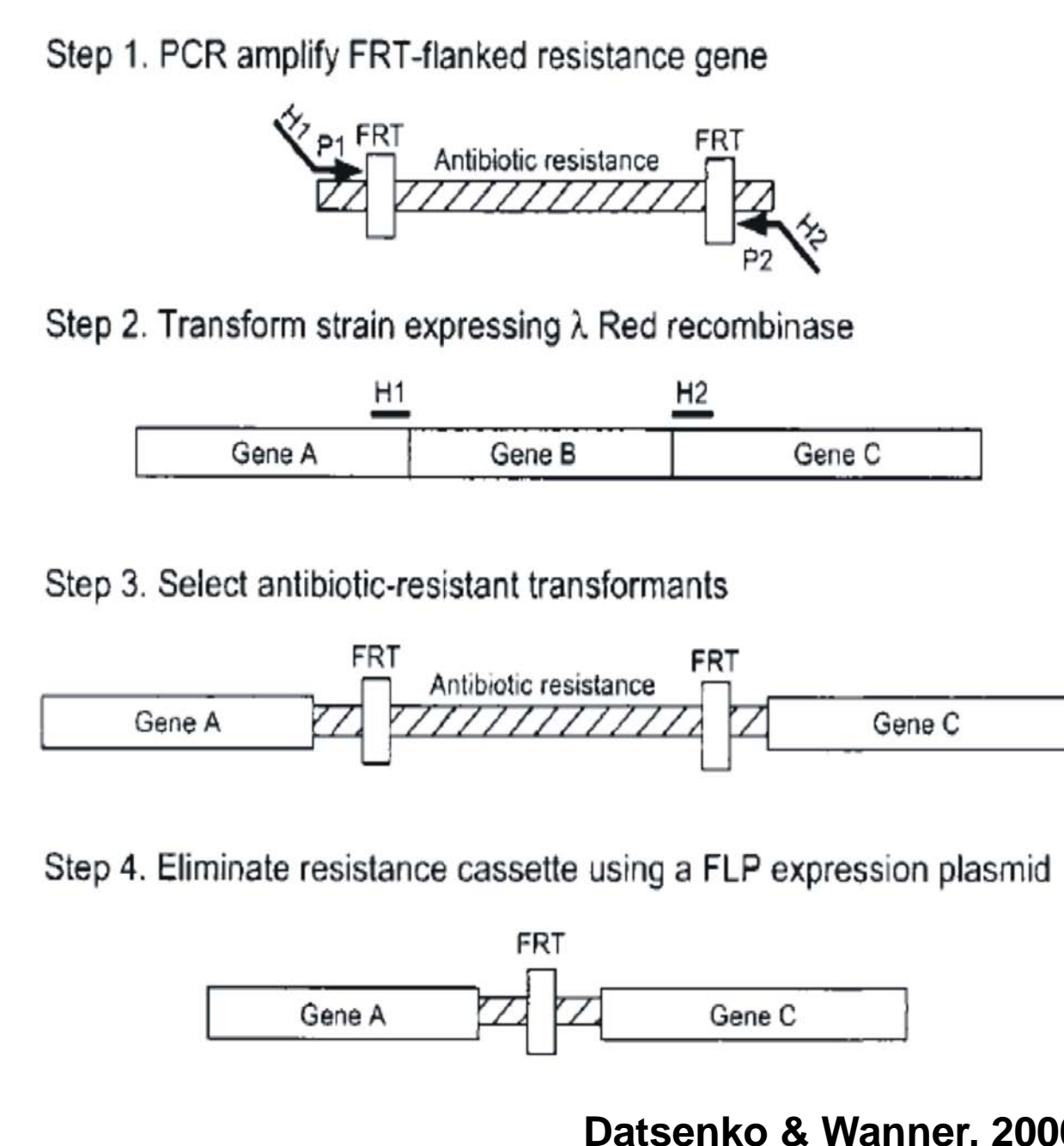
METHODS

1. Identify genes involved in attachment to meat

- Genes are selected from literature studies and expression data from gel cassette system (KU-Life)
- Selected genes:
 - flagellar genes (*fli*, *flj*, *flh*, *flg*, *mot*)
 - fimbriae and curli genes (*agf*, *fim*, *pef*, *lpf*, *std*)
 - genes related to extracellular matrix (*prg*, *bap*, *mis*, *shd*)
 - genes involved in cellulose biosynthesis (*yhj*, *bcs*)

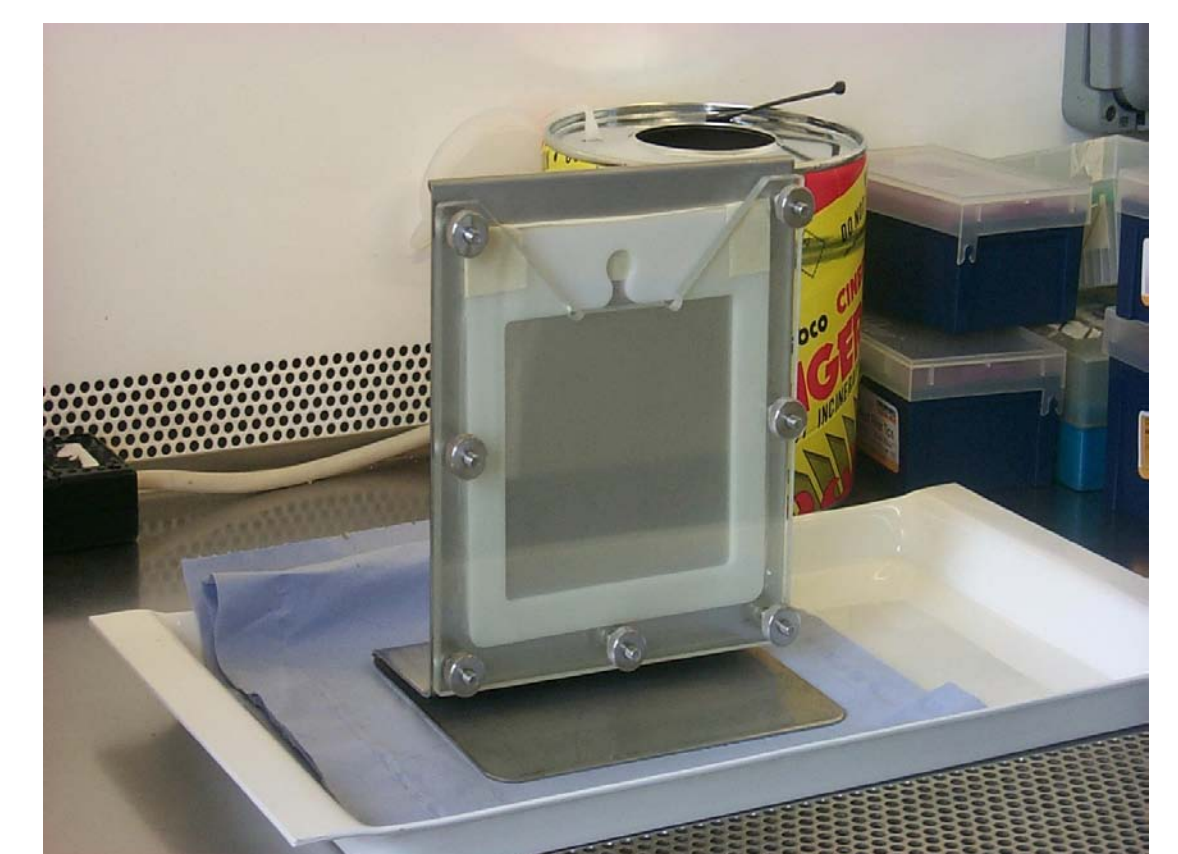
- Knock-out mutants are constructed using lambda red system (Datsenko & Wanner, 2000) and P22 transduction
- Strain: *S. Typhimurium* 4/74

- The ability to attach to meat for mutant cells and wild type cells is compared



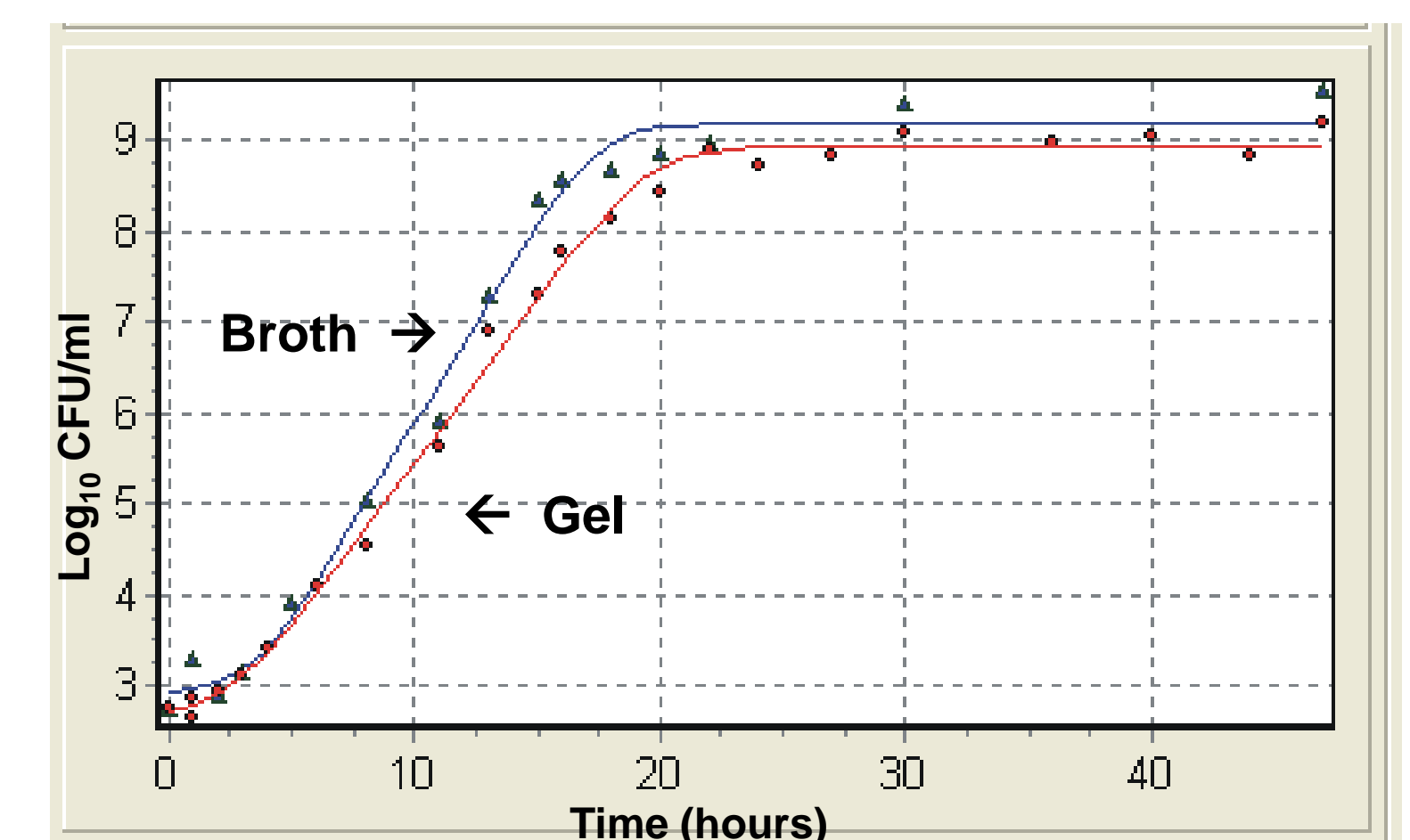
2. Attachment studies – "history of cells"

- Influence of physiological state of *Salmonella* on adhesion is studied:
 - Liquid state – broth
 - Immobilized state – gel cassette
- Gel cassette system developed by IFR (Brocklehurst et al., 1995)



Gel cassette ready for inoculation medium (IFR)

- Growth experiments in broth + gel:
- Temperature: 25 °C
- Medium (gel): LB + 22% pluronic
- Medium (broth): LB

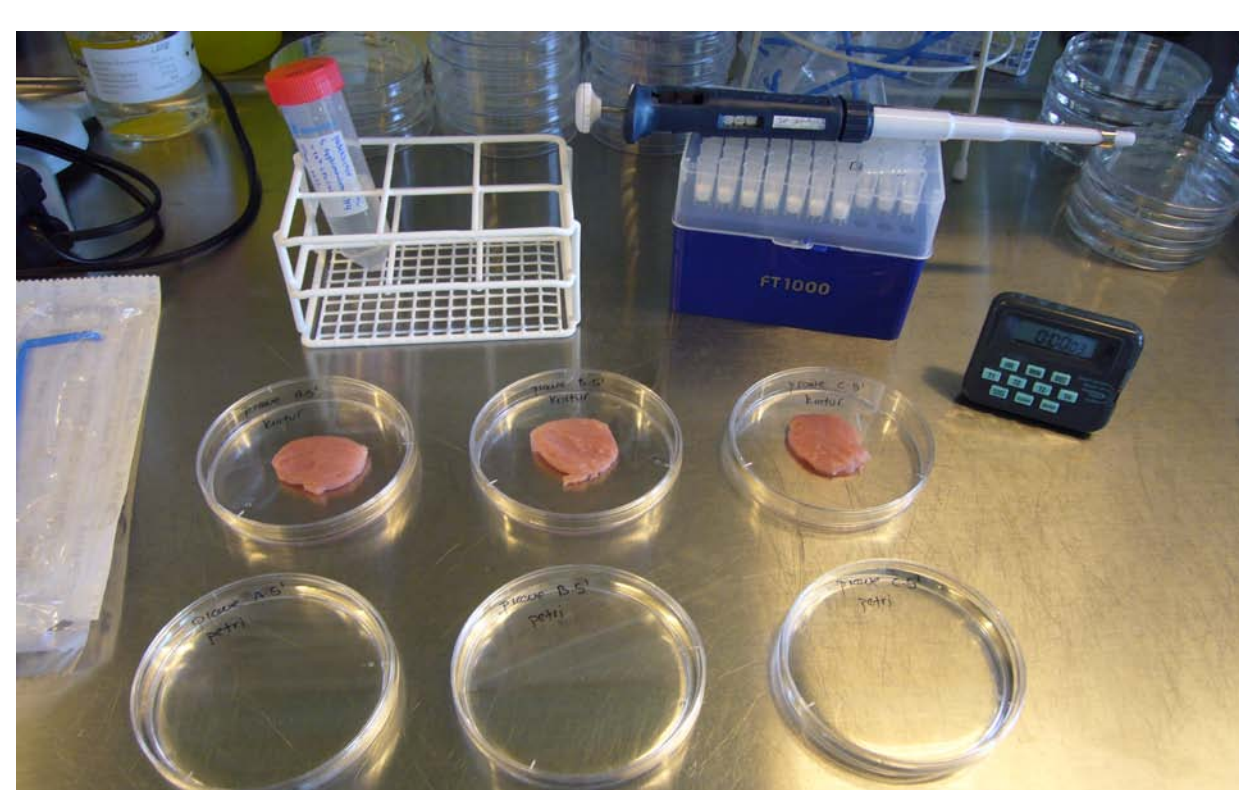


Growth curves for *S. Typhimurium* 4/74

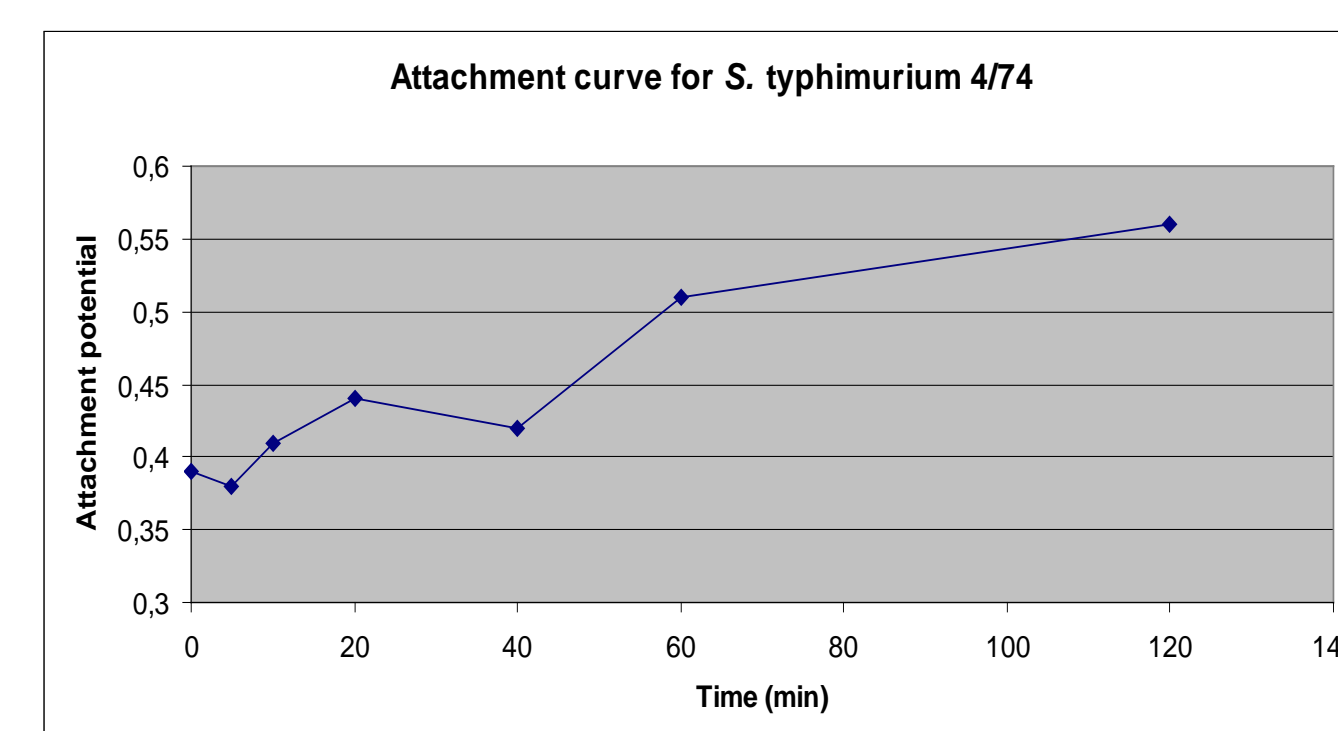
- Directly from broth and gel cassette cells are applied to a meat model at the same inoculation level (~ 10⁸ CFU/ml)

3. Attachment studies – pork meat surface model

Salmonella cells from broth and gel are diluted ten times in sterilized maximum recovery diluent (MRD) and inoculated onto pieces (9.6 cm²) of pork fillets (meat model)



Meat model



Initial test study of attachment potential of *Salmonella* to pork meat

4. Time-course of gene expression on meat

- Isolate RNA from cells strongly attached to meat over time:
 - Qiagen RNeasy mini kit using combined enzymatic lysis and Proteinase K digestion
 - Quality check using Bioanalyzer
 - Conversion of total RNA into cDNA
 - Preparing for expression analysis either by whole genome microarray technology or by quantitative RT-PCR
 - Genes of interest: attachment, virulence and lag-phase

- Optimization needed:
 - Minimize background flora?
 - Increase amount of cells extracted from the meat surface?



Microarray lab at National Food Institute, DTU

| Inoculation level (CFU/ml) | Strain | Adhesion time (min) | Temperature | Treatment | Attachment potential |
|--|---|-----------------------|-------------|---|--|
| ~ 10 ⁷ 500 µl spread on meat surface | <i>S. Typhimurium</i> 4/74: wt vs. mutant gel vs. broth | 0, 5, 10, 30, 60, 120 | Room temp. | Loosly attached: wash in 40 ml MRD for 1 min Strongly attached: stomach in 40 ml MRD for 1 min | Calculated as: <u>Strongly</u> Strongly+Loosly |

REFERENCES

Brocklehurst TF, et al. (1995). JFM 27:45-60.
 Datsenko KA & Wanner BL (2000). PNAS 97:6640-6645.